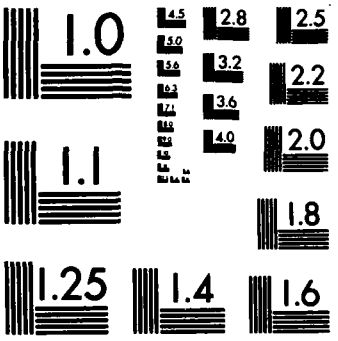
AD-A189 814 COCHLEAR HAIR CELL ELECTROCHEMISTRY: MECHANISMS FOR BIDIRECTIONAL TRANSDUCTION(U) JOHNS HOPKINS UNIV BALTIMORE MD SCHOOL OF MEDICINE W E BROWNELL 30 DEC 87 1/1

UNCLASSIFIED N00014-87-K-0037 F/G 6/1 NL



8a. NAME OF FUNDING / SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
Office of Naval Research	ONR	N00014-87-K-0037 P 00001

8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS			
	PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
800 N. Quincy Street Arlington, VA 22217-5000	61153N	RR04108	441K704	

11. TITLE (Include Security Classification)

(U) Cochlear Hair Cell Electrochemistry: Mechanisms for Bidirectional transduction

12. PERSONAL AUTHOR(S)
BROWNELL, William

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 1/1/87 TO 12/87	1987 12 30	3

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
08			outer hair cells, electromotility, electro-osmotic, laminated cisternal membranes, cable properties, polyamines, lectins

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Our objective is to determine the cellular mechanism responsible for cochlear outer hair cell (OHC) electromotility. Experiments are designed to test the working hypothesis that electrically evoked movements in OHCs result from electo-osmotic movement of cytoplasm in the outer hair cell's laminated cisternal system. More specifically, we postulate that intracellular potential gradients associated with acoustic transduction drive intracellular fluids through an electo-osmotic "pump" formed by the plasma and morphologically unique laminated cisternal membranes of the outer hair cells. The cell's cable properties are being measured to determine the range of intracellular potential gradients possible within OHCs. The effect of temperature in the mechanical frequency response is being measured. The nature of the membrane surface charge is being probed with lectin binding experiments. The possibility of interaction between polyamines and the electrical double layer is being assessed.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	(U)

22a NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Dr. J.A. Majde	202/696-4055	ONR

R&T CODE: 4410001
DATE: 30-December-1987

ANNUAL PROGRESS REPORT ON CONTRACT N00014-87-K-0037

PRINCIPAL INVESTIGATOR: William E. Brownell, Ph.D.

CONTRACTOR: The Johns Hopkins University School of Medicine

CONTRACT TITLE: Cochlear Hair Cell Electrochemistry: Mechanisms for Bidirectional Transduction

START DATE: 1 January 1987

RESEARCH OBJECTIVE: To determine the cellular mechanism responsible for outer hair cell (OHC) electromotility. Our working hypothesis is that the electrically evoked movements of OHCs result from electo-osmotic movement of cytoplasm in the cell's laminated cisternal system. More specifically, we postulate that intracochlear potential gradients associated with acoustic transduction drive intracellular fluids through an electo-osmotic "pump" formed by the plasma membrane and the morphologically unique laminated cisternal membranes.

PROGRESS (Year 1):

1. MORPHOLOGICAL EXAMINATION OF MEMBRANE SURFACE CHARGE: We have explored the nature of the outer hair cell's membrane coat by utilizing the lectins FITC-WGA and FITC-HPA to identify specific membrane associated carbohydrates. Wheat germ agglutinin (WGA) bound with the cell coat of both inner and outer hair cells (IHC & OHC) suggesting the presence of either N-acetyl-D-glucosamine or sialic acid. In contrast, glycoconjugates with terminal N-acetyl-D-galactosamine residues that bind with helix pomatia agglutinin (HPA), were demonstrated inside the plasma membrane of outer hair cells. WGA and HPA lectin binding implies the presence of anionic glycoconjugates that furnish added negative charge on the membranes to which they are fixed. If the intracellular binding of HPA indicates a strongly polyanionic surface charge on the membranes of the intercisternal spaces, the resulting surface potential could increase the magnitude of the electrokinetic events postulated to be associated with OHC electro-motility.

2. MEASURING AXIAL POTENTIAL GRADIENTS IN OHC: Our working hypothesis requires that axial potential gradients exist in the outer hair cells. We are measuring the cable properties of the outer hair cell to determine if potential grandients of sufficient magnitude can be maintained by the cell. Two patch electrodes are placed on the cell at locations near one end and the middle of the cell. An intracellular electrode in then inserted at the opposite end. Current and voltage are measured at each electrode while current pulses are alternately injected at each of the three electrodes. These measures permit a characterization of the length constant associated with the cells. Preliminary evidence indicates the cells membrane properties result in axial potential gradients when the cell is electrically stimulated.

3. POSSIBLE INVOLVEMENT OF POLYAMINES IN HAIR CELL TRANSDUCTION: The anti-neoplastic drug, difluoromethylornithine (DFMO), has caused reversible sensori-neural hearing loss in clinical tests on humans. DFMO is a specific blocker of the enzyme ornithine decarboxylase and blocks the production of polyamines. We have developed a guinea pig animal model that mimics the reversible hearing loss seen in humans. The highly polycationic nature of the polyamines suggest their possible involvement in electrokinetic phenomena.

4. SETTING UP PHOTOMETRIC SYSTEM: We have also made progress in fabricating an optical device to measure high frequency, low displacement movements of hair cells. The image of electrically stimulated outer hair cells is projected out of the microscope onto a linear position detector. The detector's output is differentially amplified and either signal averaged or fed into a lock-in analyzer prior to signal averaging.

WORK PLAN (Year 2):

1. MICROPHOTOMETRICALLY CHARACTERIZE THE DYNAMICS OF OHC CELL SHAPE CHANGES in response to step, pulse and sinusoidal electric stimulation. By measuring the movement magnitude and phase we will establish mechanical frequency response properties. These experiments will establish normative data with which to compare experimental results. Our potential gradient measures will be extended as a complete discription of an electrokinetic response will require an accurate determination of the potential gradient driving the response. The use of voltage sensitive dyes and vibrating probes to facilitate measuring intracellular potential gradients will be explored.

2. MEASURE THE EFFECT ON OHC ELECTROMOTILITY OF MANIPULATIONS THAT CAN AFFECT ELECTRO-OSMOSIS: Manipulations include administration of substances capable of modifying the cell surface charge and parametrically varying the ionic composition of the bathing media. We will use aminoglycosides, polyamines and test hair cells taken from DMFO treated animals. Changes in temperature should produce a change in OHC dynamics. The equation describing the velocity of movement in electro-osmosis is:

$$\vec{v} = \frac{\vec{E}\epsilon\zeta}{4\pi\eta}$$

where $\vec{v}$ is the velocity of fluid flow
$\vec{E}$ is the potential gradient
ϵ is the dielectric constant of the medium
ζ is the zeta potential
η is the viscosity of the moving fluid.

The equation describes electro-osmotically driven fluid velocity as being inversely proportional to viscosity. For a liquid, viscosity is roughly related to temperature by:

$\eta = Ae^{\frac{\beta}{T}}$; so that electro-osmotic fluid velocity is: $\vec{v} \propto \left[Ae^{\frac{\beta}{T}}\right]^{-1}$

This relationship is postulated to describe the flow of cytoplasm between the membranes of the laminated cisternae in response to a potential gradient, which, in turn, may drive pressure changes within the cell, generating the conformational changes we detect as movement. While the above equation does not describe the velocity of the cell movement we will record, changes in electro-osmotic flow caused by temperature should elicit proportional changes in movement if other variables remain constant. Experimental data collected on the temperature dependence of muscle contraction have shown about a six fold change in contraction velocity over the same temperature range we will be using in our protocol. Expected electro-osmotic velocity changes should be about two fold over the same range. A measurable difference in the velocity of movement will prove a valuable test of our working hypothesis.

3. DETERMINE THE CELLULAR LOCALIZATION OF HPA LECTIN BINDING. HPA lectin, colloidally bound with gold or horseradish peroxidase will be used to determine where the HPA lectin binds within the cell using transmission electro-microscopic techniques. This work will be done in collaboration with Drs. Pablo Gil-Loyzaga (Madrid) and Peter Santi (Associate Professor, U. Minnesota).

INVENTIONS: None. No potentially patentable devices.

PUBLICATIONS AND REPORTS (Year 1):

1. A manuscript authored by Gil-Loyzaga, P.E., and Brownell, W.E., entitled "Wheat germ agglutinin and Helix Pomatia lectin binding on cochlear hair cells" has been accepted for publication in Hearing Research

Book chapters

2. An abstract authored by Jansen, C.J., Mattox, D.E., Miller, K.D., and Brownell, W.E., entitled "An animal model of hearing loss from alpha-difluromethylornithine (DFMO)", will be published in the Abstracts of the Midwinter Research Meeting of the Association for Research in Otolaryngology 11 (1988).

3. An abstract authored by Zidanic, M., and Brownell, W.E., entitled "Two-dimensional analysis of cochlear microphonics in the guinea pig cochlea",will be published in the Abstracts of the Midwinter Research Meeting of the Association for Research in Otolaryngology 11 (1988).

4. Seminars entitled, "Electro-kinetic basis for outer hair cell shape changes" were presented at: Committee on Neurobiology, University of Chicago, Chicago, 30-April-1987; and the Department of Cell Biology and Biophysics, Unverisity of Illinois, Medical School, Chicago, 1-May-1987.

5. The Joseph Bettingen Invited Lecture in Otolaryngology, entitled, "Implications of Outer Hair Cell Motility for Hearing" was presented at: Department of Otolaryngology, University of Minnesota, Minneapolis, 4-May-1987.

6. The seminar entitled, "Outer hair cells dance to the tune of an intracochlear potential gradient", was presented at: The Kresge Hearing Research Laboratory, Ann Arbor, Michigan, 8-October-1987.

TRAINING ACTIVITIES: An undergraduate, graduate, and two Otolaryngology Head&Neck Surgery residents have participated in portions of the research. A post-doctoral fellow and another resident will be soon be joining the laboratory.

Women or minorities - 3
Non-citizens - 1 (citizen of France - will be a 2 post-doctoral fellow in 88)

END
DATE
FILM
4-88
DTIC